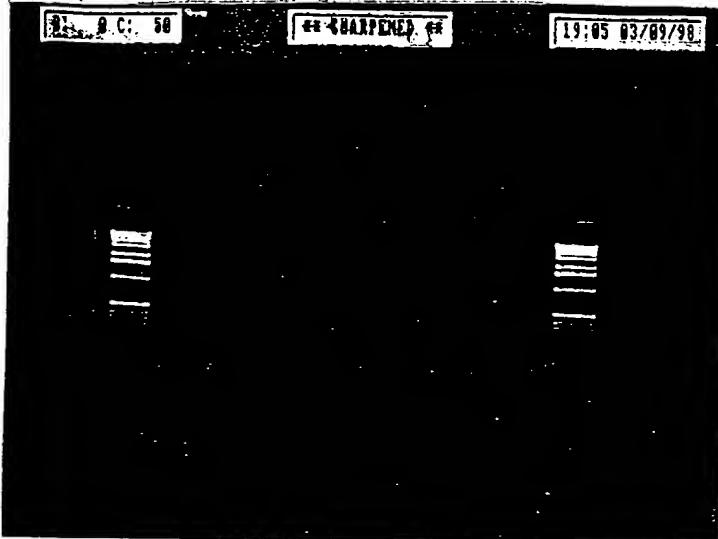
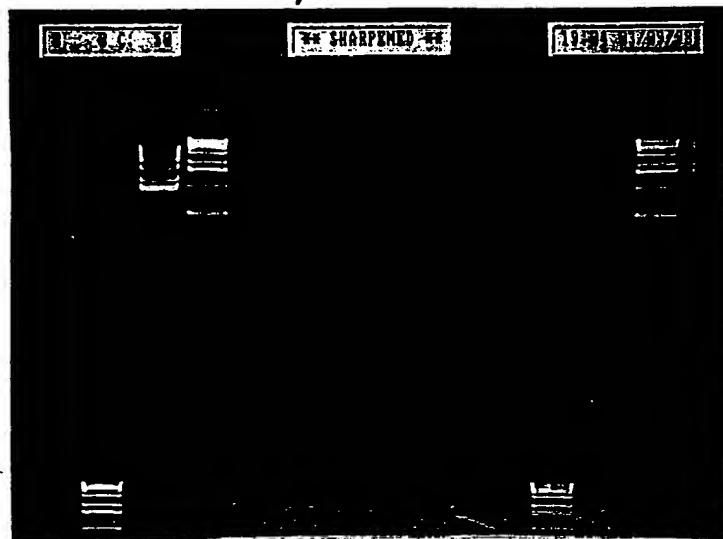


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49665
200bP



Thoughts try DMSO w/amplifag and see if this helps.

sourcing for 49665.

18

- 1> 100ng sslib
- 90 5> Klentag buffer
- 18 > dNTP
- 18 > forward
- 18 1> reverse
- 18 1> Klentag
- 720 40> ddH₂O
- 50> Totac

95°C 5 min 1 cycle

95 1 min
57 1 min
72 1.5 min
20 cycles

1.5% TAE gel

728 may possibly have the band

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Bethany Deul

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PCR sourcing w/ 5% DMSO + tag

20

1x ss. ^G lil	
5x buffers	1100
3x MgCl ₂	600 660
2.5x DMSO	55
1x forward	220
1x reverse	220
1x dNTP	220
1x ampliTaq	220
To 50x ddH ₂ O	759

95°C 5 min 1 cycle

95°	1 min
50°	1 min
72°	1 min 25 cycles

product should be around 200 bp

277 278 294 possibilities

versus gel and do Southern
order

278 from yesterday	2790
26	229
94	230
99	247
135	247
153	255
154	243
22710	294
227	301
228	302
22912	
229	

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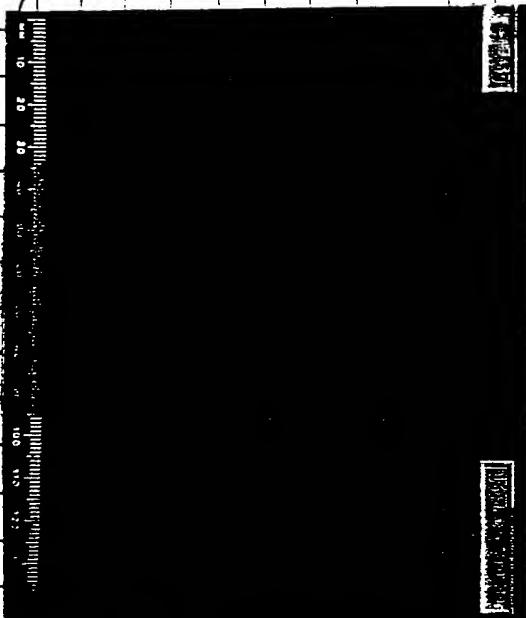
Date

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Barthanne Denel

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justamps add 500₁ transfection + 500₂ congs
let rock 1 hr add 10 ml media

1 56436
5 571095
9 56350
15 571094
17 57834
4 58800

let go until Friday
then harvest cells.

Gibson gets Southern for 49165 cytokine.
Probably 227 or 228 has it
If not try 254 etc

Take apart southern. Neutralize and leave
2-3 hrs in 80° vacuum oven

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PAGE: 1

USER: 5 ID: 32P MAXI COMMENTS:
 PRESET TIME: 1.00 H#: YES SAMPLE REPEATS: 1 DATA CALC: CPM
 PRINTER: STD SCR: NO REPLICATES: 1 COUNT BLANK: NO
 RS232: OFF RCM: YES MULTIPLIER: 1.000000 VIAL SIZE: MAXI

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE: YES

SAM NO	POS	TIME MIN	H#	32P CPM	RCM	ELAPSED TIME
1	1-1	0.51	49.0	86387.21	0.95	0.00 1.38

make probe 49665

1x 49665³²P

10x \times^{32} P ATP

10x T4 pk buffer

2x T4 pk

41x ddH₂O

mix and incubate 30 min 37°C

clean

dilute 1:10 and count

Miniprep + and do Xba digest

5x CDNA

2x buffer HNM402

1x Xba

12x ddH₂O

mix

32

164

32

384

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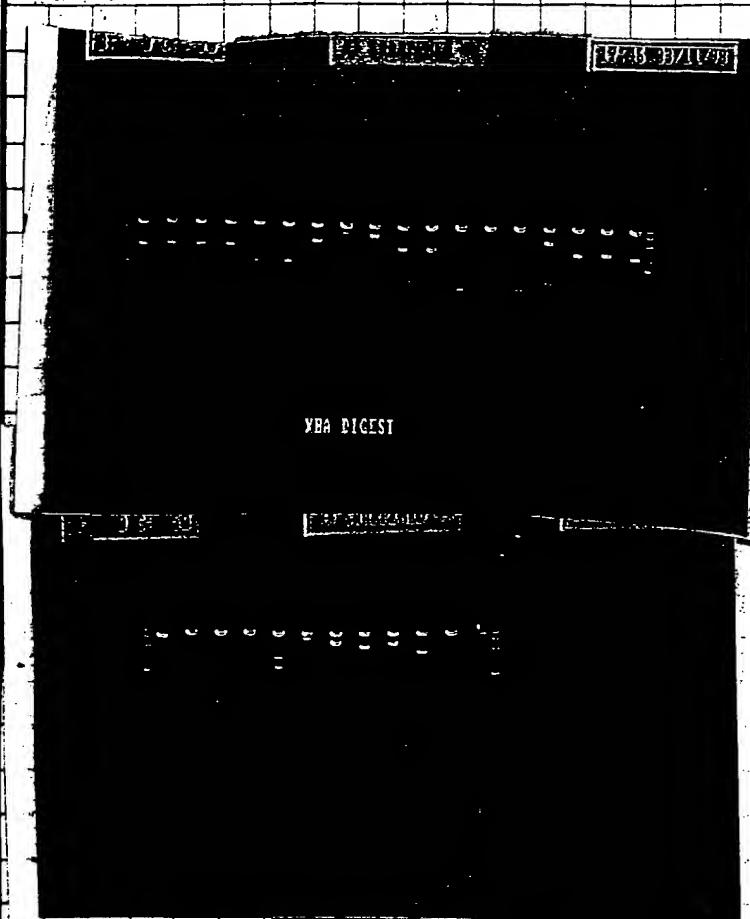
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From Page N



52159 (3) ✓
 (4)
 (5)
 (7) any

52721 3
 (4)
 (5)

52165 2 (M)
 (3)

52163 (7) any
 (3)

52175 (1) any
 (3)
 (4)

52035 1 ?
 (2)
 (4)
 (5) any

52161 1
 (2)
 (7)

52777 (4) ?
 (5) - ? replace perso

52765 (1)
 (2)
 (3)

52162 (1) o replace

52160 1

42836 2 → ?

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Date

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Barbara Dene

Dat

R c rded by

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Wash. Southern

2XSSC + 0.1% SDS

42°

40 min

0.5XSSC + 0.1% SDS

42°

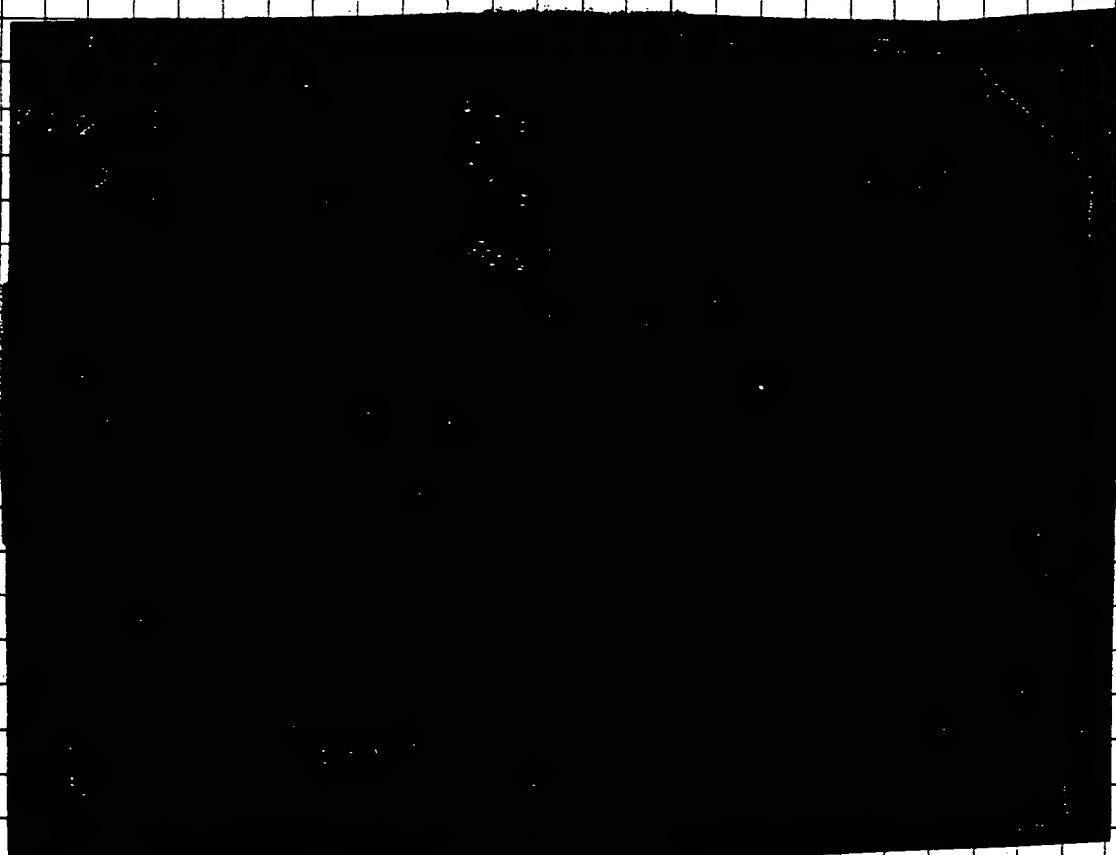
15 min

0.1XSSC + 0.1% SDS

42°

10 min

Expose to film 2 hrs develop



Source for cloning 19665
 27, 228, 294

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184? 247? 255?

38237 1034

38085 - 2967 462

60684 150

Cloning

→ 491665 - wash hydros

2XSSC + 0.1% SDS 40 min

0.5XSSC + 0.1% SDS 20 min

late air dry expose to films develop

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Sourcing
rerun PCR to check size of
601684
the bottom bands
are 150

so use 261, 153, 254, or 247
for cloning

Cloning - 1 pick + for 49665
grow O/N
39 + 26 + 65 + in 17,000 colonies

2) transform and plate rescues from yesterday

PCR for new sourcing

60748 - 16 1.6 51^o annealing

38085 - 8 0.1r 12 diff annealing temp

95^o 5 min 1

95^o 1 min

57 - 63^o 1 min

72^o 1 min 10 sec 25

72^o 5 min 1

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do minipreps on 49665

Baculovirus

set up 1stamps

Cloning

make probes for

52781

36456

36967

10974

0.5uL probe

12

501084

38096

3uL pnk buffer

36

60748

36461

5uL x³²P ATP

60

38085

35735

2uL T4 pnk

24

38237

36963

19.5uL ddT₂ 0

234

-

clear probes in Puricle quant G⁵⁰ micro columns

freeze

Set up PCR for synthesis of 14 oligos in 12 librariesplate in vivo from yesterday

Set up PCR for reverse of 49665

12 oligos

12

5uL Thermo buffer

-

cycling

1uL forward 49665

-

cond

1uL reverse 49665

-

for

1uL dNTP

-

NORTH

1uL Thermo

-

except

40uL ddH₂O

-

72°C 1.5 min

95°C 5 min 1 cycle

95°C 1 min

95°C 1 min

55°C 1 min

59°C 1 min

72°C 3 min

72°C 3 min 3 cycles

20 cycles

95°C 1 min

57°C 1 min

72°C 3 min

3 cycles

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49665 P2N

Set up Xba Digest for 49665

5x	CDNA	12
2x	React 2	24
1x	Xba I	12
12x	ddH ₂ O	144

Set digest like 37°C

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Witnessed & Understood by me,

Date

Invented by

Date

Bethanne Dovel

Recorded by